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Amendments to the Claims

Please amend Claims 23, 25-28, 59, 62, 66 and 67. The Claim Listing below will replace all prior versions of the claims in the application:

Claim Listing

1 - 22. (Canceled)

23. (Currently Amended) A method for identifying genes that carry one or more alleles that confer an increased likelihood ~~risk~~ of mortal disease, comprising

   a)   determining the number of two or more ~~all~~ detectable point mutations in a gene or gene segment in a population of young individuals, and calculating the sum of the frequencies of all point mutations determined for each gene or segment;

   b)   determining the number of a plurality of ~~all~~ detectable point mutations in a gene or gene segment in a population of aged individuals and calculating the sum of the frequencies of the ~~all~~ point mutations determined for each gene or segment;

   c)   comparing the sum of the frequencies of point mutations that are found in a selected gene or portion thereof of the young population calculated in a) with the sum of the frequencies of point mutations that are found in the same gene or portion thereof of the aged population calculated in b),

wherein a statistically significant decrease in the sum of the frequencies of point mutations associated with a particular gene in the aged population indicates that said selected gene carries one or more alleles that confer an increased likelihood ~~risk~~ of mortal disease.

24. (Canceled)

25. (Currently Amended) A method for identifying genes that carry a harmful allele, comprising:

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a)   identifying the set of point mutations that are found in one or more genes or
      portions thereof of a population of young individuals, wherein the set comprises
      all <u>detectable</u> point mutations occurring at a frequency at about or above 5×10^{-5}
      in a population from which the sample was obtained, and determining the
      frequency with which each point mutation occurs;

b)   identifying the <u>detectable</u> set of point mutations that are found in the genes or
      portions thereof of a population of aged individuals, and determining the
      frequency with which each point mutation occurs; and

c)   comparing the frequency of each point mutation identified in a selected gene or
      portion thereof of the young population determined in a) with the frequency of the
      same point mutations identified in said selected gene of the aged population
      determined in b),

wherein a significant decrease in the frequency of two or more point mutations in said
selected gene of the aged population relative to said selected gene of the young
population indicates that said selected gene carries a harmful allele.

26.   (Currently Amended) The method of Claim 25 further comprising:

d)   determining the frequency of <u>a subset of the mutations identified in c) that are</u>
      <u>significantly decreased in the aged population</u> ~~total mutations~~, wherein the <u>subset</u>
      <u>of point</u> mutations are ~~point mutations of total nonsynonomous point mutations;~~
      ~~or total~~ obligatory knockout point mutations; ~~or any combination thereof, wherein~~
      ~~the mutations are significantly decreased in the aged population determined in c)~~
      ~~in one or more intermediate age-specific populations;~~

e)   determining the age-specific decrease in frequency of any of said <u>subset</u> ~~sets~~ of
      point mutations; and

f)   comparing the age-specific decrease in frequency with the expected age-specific
      decrease in frequency of a set of harmful alleles that cause of a particular mortal
      disease,

wherein a determination that the age-specific decrease in frequency determined in e) is
not significantly different from the expected age-specific decrease in frequency of

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harmful alleles further indicates that said selected gene carries a harmful allele and has a high probability of being causal of said mortal disease.

27.   (Currently Amended) The method of Claim 25 ~~26~~ further comprising:

d)   identifying a subset of obligatory knockout point mutations in the point mutations identified in c) as being decreased in the aged population:

e) ~~g)~~   determining the frequency of the point ~~total~~ mutations identified in d), ~~wherein the mutations that are point mutations of total nonsynonomous point mutations, or total obligatory knockout point mutations; or any combination thereof; and wherein the mutations are~~ significantly decreased in ~~the aged population in said selected gene of~~ one or more proband populations, wherein the ~~a~~ proband population consists of persons diagnosed with the mortal disease drawn from a common ~~the same~~ population; and

e) ~~h)~~   comparing the frequencies of the subset of point ~~said total~~ mutations in said selected gene or portion thereof in the young population with the frequencies of said subset of ~~two or more~~ point mutations in said selected gene or portion thereof in the one or more proband populations,

wherein a significant increase in the frequencies of said subset of ~~one or more~~ point mutations in the proband population relative to the young population indicates that said gene carries one or more harmful alleles that confer risk for said disease.

28.   (Currently Amended) The method of Claim 27 ~~26~~, wherein the proband population consists of individuals with early onset disease. ~~further comprising:~~

~~g)   determining the frequency of said total mutations, wherein the mutations are point mutations of total nonsynonomous point mutations, or total obligatory knockout point mutations, or any combination thereof, and wherein the mutations are significantly decreased in the aged population in said selected gene of one or more proband populations consisting of individuals with early onset disease; and~~

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~~h)   comparing the frequencies of said total mutations in said selected gene or portion~~
~~thereof in the young population with the frequencies of said two or more point~~
~~mutations in said selected gene or portion thereof in the proband populations;~~
~~wherein a significant increase in the frequencies of said one or more point mutations in~~
~~the proband population relative to the young population indicates that said gene carries at~~
~~least one harmful allele that is a secondary risk factor.~~

29 - 58.   (Canceled)

59.   (Currently Amended) A method for ~~of~~ identifying ~~total mutations, wherein the mutations~~
~~are point mutations of total nonsynonomous point mutations, or~~ total detectable
obligatory knockout point mutations, ~~or any combination thereof,~~ in any target region of a
genome of a population, wherein said point mutations cause or accelerate the appearance
of a mortal disease or prevent or delay the appearance of a mortal disease, comprising:

a)   separately determining, ~~in each subpopulation,~~ the frequencies ~~set~~ of all detectable
      mutations occurring at a frequency at or above 5×10^{-5} in members of a ~~the same~~
      population that comprises subpopulations selected from the group consisting of
      young, aged, intermediate age, afflicted with disease, afflicted with a disease of
      early age onset and afflicted with a disease of late age onset; and

b)   determining the frequencies of each mutation of a) within one or more ~~the~~
      subpopulations,

      wherein a decrease in the frequency in the aged population compared to the young
      population is indicative of an allele that causes or accelerates a mortal disease, and an
      increase in frequency in the intermediate or aged population compared to the young
      population is indicative of an allele that prevents or delays the appearance of a mortal
      disease.

60.   (Previously presented) The method of Claim 25, wherein said point mutations are
       identified using a method that comprises:

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a) providing a first pool of DNA fragments comprising a gene or portion thereof, wherein said pool is isolated from a population and contains DNA pooled from 10 to 10,000 individuals;

b) amplifying a target region of said gene or portion from each of said fragments in a high fidelity polymerase chain reaction (PCR) under conditions suitable to produce double-stranded DNA products that contains, or may be subsequently attached to, a terminal high temperature isomelting domain that is labeled with a detectable label, and wherein the mutant fraction of each PCR-induced mutation among the PCR products is not greater than about 5×10^{-5} for a pool created by DNA from 10,000 persons;

c) melting and reannealing the product of b) under conditions suitable to form duplexed DNA, thereby producing a mixture of wild-type homoduplexes and heteroduplexes that contain point mutations;

d) separating the heteroduplexes from the homoduplexes based upon the differential melting temperatures of said heteroduplexes and said homoduplexes and recovering the heteroduplexes, thereby producing a second pool of DNA that is enriched in target regions containing point mutations;

e) amplifying said second pool by high fidelity PCR under conditions where only homoduplexed double-stranded DNA is produced, thereby producing a mixture of homoduplexed DNA containing wild-type target region and homoduplexed DNAs that contain target regions that include point mutations;

f) resolving from step e) hetero-duplexed DNAs containing target regions that include point mutations based upon the differential melting temperatures of the DNAs, and recovering the resolved DNAs that contain a target region that includes point mutations; and

g) determining the sequence of the target region of the recovered DNAs to identify point mutations within the target region.

61. (Canceled)

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62.   (Currently Amended) A method for identifying genes that carry one or more harmful
       alleles, comprising:

       a)   identifying in one or more samples two one or more point mutations that are
             found in one or more genes or portions thereof of a population of young
             individuals, determining the frequency with which each point mutation occurs,
             and calculating the sum of the frequencies of all point mutations identified for
             each gene or segment;

       b)   identifying in one or more samples two one or more point mutations that are
             found in one or more genes or portions thereof of a population of aged
             individuals, determining the frequency with which each point mutation occurs,
             and calculating the sum of the frequencies of all point mutations identified for
             each gene or segment;

       c)   determining the subset of point mutations that are obligatory knock-out point
             mutations for steps a) and b); and

       d)   comparing the sum of the frequencies of obligatory knock-out mutations that are
             found in a selected gene or portion thereof of the young population calculated in
             a) with the sum of the frequencies of obligatory knock-out point mutations that
             are found in the same gene or portion thereof of the aged population calculated in
             b),

       wherein a significant decrease in the sum of the frequencies of obligatory knock-out point
       mutations in the aged population indicates that said selected gene carries one or more
       harmful alleles.

63. through 65.   (Canceled)

66.   (Currently amended) A method for identifying genes that carry one or more alleles that
       confer an increased likelihood risk of mortal disease, comprising:

       a)   determining the number and nucleotide sequence identities of all detectable point
             mutations in a gene or gene segment in a population of young individuals,
             wherein said gene segment consists of the exons and splice sites of genes that

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code for proteins, wherein detectable point mutations are <u>selected from the group</u> <u>consisting of:</u> ~~nonsynonomous such as~~ <u>point mutations resulting in</u> amino acid substitutions, small insertions or deletions, termination codons or splice site mutations, in a population of young individuals, and calculating the sum of frequencies of all point mutations determined for each gene or segment;

b)   determining the number and nucleotide sequence identities of all detectable point mutations in a gene or gene segment in a population of aged individuals, wherein said gene segment consists of <u>the</u> exons and splice sites of genes that code for proteins, wherein detectable point mutations are <u>selected from the group</u> <u>consisting of:</u> ~~nonsynonomous such as~~ <u>point mutations resulting in</u> amino acid substitutions, small insertions or deletions, termination codons or splice site mutations, and calculating the sum of the frequencies of all point mutations determined for each gene or segment; <u>and</u>

c)   comparing the sum of the frequencies of the specified type or types of ~~nonsynonomous~~ point mutations that are found in a selected gene or segment thereof of the young population calculated in a) with the sum of the frequencies of the specified type or types of ~~nonsynonomous~~ point mutations that are found in the same gene or segment thereof of the aged population calculated in b),

wherein a statistically significant decrease in the sum of the frequencies of the specified type or types of ~~nonsynonomous~~ point mutations associated with a particular gene in the aged population indicates that said selected gene carries one or more alleles that confer <u>an</u> <u>increased likelihood</u> ~~risk~~ of mortal disease.

67.   (Currently amended) A method for identifying genes that carry one or more alleles that confer <u>an increased likelihood</u> ~~risk~~ of mortal disease, comprising

a)   determining the number and nucleotide sequence identities of all detectable point mutations in a gene or gene segment in a population of young individuals, wherein the gene segment consists of the exons and splice sites of genes that code for proteins and enumerating the detectable point mutations that are expected to result in a nonfunctional gene product such as with small insertions or deletions;

termination codons or splice site mutations, or any combination thereof, and calculating the sum of the frequencies of all such specified type or types of point mutations determined for each gene or segment;

b) determining the number and nucleotide sequence identities of all detectable point mutations in a gene or gene segment in a population of aged individuals, wherein the gene segment consists of the exons and splice sites of genes that code for proteins and enumerating the detectable point mutations that are expected to result in a nonfunctional gene product such as with small insertions or deletions, termination codons and splice site mutations, and calculating the sum of the frequencies of all such specified type or types of point mutations determined for each gene or segment;

c) comparing the sum of the frequencies of specified types of point mutations that are found in a selected gene or segment thereof of the young population calculated in a) with the sum of the frequencies of specified type or types of point mutations that are found in the same gene or segment thereof of the aged population calculated in b),

wherein a statistically significant decrease in the sum of the frequencies of the specified type or types of point mutations associated with a particular gene in the aged population indicates that said selected gene carries one or more alleles that confer risk of mortal disease.

68. (Previously presented) The method of Claim 25 wherein said point mutations are identified by amplifying DNA obtained from the individual members of the population samples and determining the nucleotide sequence of the DNA.

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